Enrichment and isolation of anaerobic thermophilic cellulolytic bacteria from mulch

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1. ABSTRACT

Cellulose is the major component of lignocellulose biomass. It represents the single largest bioenergy resource on our planet, and it has been looked upon to provide much of the renewable bioenergy we can efficiently harness. All biotechnologies to convert cellulosic biomass to biofuels require the hydrolysis of lignocellulose to sugars (glucose and cellubiose). Cellulases from bacteria and fungi are commonly used to mediate cellulose hydrolysis. It is of scientific and biotechnological interest to find more efficient and robust cellulolytic microorganisms and cellulase. The objective of this study was to isolate thermophilic anaerobic bacteria that can rapidly hydrolyze cellulose. A mulch sample was collected from a big pile of mulch and inoculated into a semi-defined medium. Ball-milled filter paper was used as the sole substrate. The culture was incubated at 55 ℃ and successively transferred into a fresh medium upon the depletion of cellulose substrate. The enriched cultures were analyzed for their products and the results showed that short chain fatty acids, mainly acetate, propionate and butyrate, made up of the main products from the digestion of cellulose. Isolation of cellulolytic bacteria was attempted using the plate overlay technique, with the top layer containing ball-milled cellulose. Single colonies that produced a surrounding clear zone were inoculated into liquid medium containing cellulose to confirm cellulolysis. 16 isolates showed complete degradation of the added cellulose at 55℃. Three isolates were identified by sequencing their 16S rRNA gene. Preliminary analysis of the retrieved sequences revealed novel species of *Clostridiaceae* and *Acetivibrio* spp. These isolates are being further characterized genetically, physiologically and biochemically. If they possess superior cellulolytic and/or fermentative feature(s), they could be very useful to harness biofuels from cellulosic biomass.

2. INTRODUCTION

2.1. The use of cellulose to generate renewable bioenergy is highly sought after.

1) Cellulose is the most common organic compound on Earth and it represents the largest renewable bioenergy resource.

2) The technologies for conversion of cellulose to biofuels need efficiency.

3) Cellulases commonly used for cellulose hydrolysis can be found in bacteria and fungi.

4) Thermophilic cellulolysisis preferable for bioenegy production.

5) Efficient and robust cellulolytic microorganisms and cellulases is of great scientific and technological interest.

2.2. Issues addressed.

1) Isolation of cellulolytic thermophilic bacteria from enriched cultures.

2) Identification of the isolates by 16S rDNA sequencing.

3) Analysis of the products from the digestion of cellulose.

3. EXPERIMENTAL

1. Enrichment from a mulch sample to select and enrich cellulose-digesting bacterial consortium.

2. Short chain fatty acids analysis by GC for the products of cellulose digestion.

3. Isolation of cellulolytic bacteria using a plate overlay technique.

4. 16S rDNA sequencing to identify the isolated bacterial species.

4. RESULTS

4.1 Enrichment culture in serum bottles

Fig. 1. The enrichment culture from a mulch sample using 0.4% ball-milled filter paper as the sole substrate. The culture was incubated at 55℃ and successively transferred into a fresh medium every 48 hours. The added cellulose was completely degraded. The bottle to the left is the control without the inoculation.

4.2 Short chain fatty acid analysis

sample	Acetate	Propionate	Iso-Butyrate	Butyrate
CS1	24.11	5.93	0.72	7.92
CS2	28.69	6.43	0.95	6.11
CS3	33.84	7.47	1.22	5.17
CS4	32.87	7.36	1.20	5.47
CS5	28.28	6.33	0.94	4.75
CS6	30.59	6.74	1.15	5.30
CS7	32.11	7.13	1.13	4.47
CS8	32.63	7.06	1.17	4.30
CS9	34.83	7.20	1.24	4.97

Fig. 2. The results for the short chain fatty acid analysis. The samples from CS1 to CS9 are the enrichment cultures successively transferred every 48 hours.

4.3 Confirmation of cellulolysis by the isolates

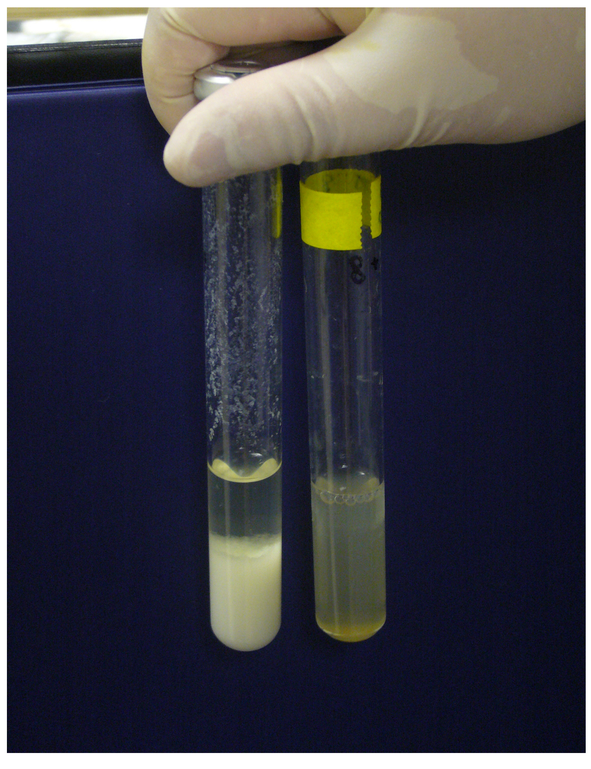

Fig. 3. Cellulose degradation by each isolates was confirmed. The tube to the right is the control without inoculation.

4.4 RFLP analysis for the 16S rDNA

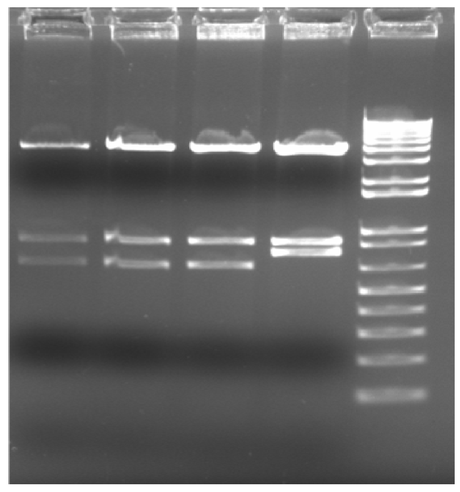

Fig. 4. RFLP analysis of recombinant plasmids carrying amplified 16S rDNA from four isolates. The plasmids were digested with *Eco* RI.

4.5 phylogenetic analysis of three of the isolates.

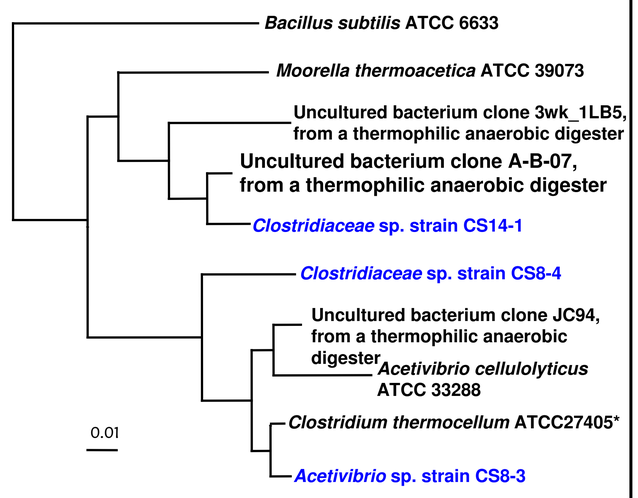

Fig. 5. A phylogenetic tree constructed from an alignment of sequences of the three isolates (shown in blue) and their most similar sequences retrieved from GenBank. Note, the *C. thermocellum* strain is placed into the unclassified *Clostridiaceae* in RDP. The scale represent 0.01% substitution.

5. SUMMARY

- **Enrichment cultures were successfully selected that effectively degrade cellulose at 55°C.**
- **The initial microbial community underwent succession.**
- **The enriched cultures could use cellulose as the sole substrate and produce acetate, propionate, butyrate, and iso-butyrate as main fermentation products.**
- **Acetate, propionate, and iso-butyrate increased while butyrate decreased over the enrichment process.**
- **The plate overlay technique is a reliable technique to selectively isolate cellulolytic bacteria.**
- **The isolated bacteria rapidly degrade cellulose.**
- **Three of the isolates appeared to be represent novel species of thermophilic cellulolytic bacteria.**

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